FORM PTO-1390 U S DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER	
50179-096	
U.S. APPLIC. NO. (if known, see 37 CFR 1.5)	
09/936216	

INTERNATIONAL APPLICATION NO	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/AU00/00181	March 10, 2000	March 10, 1999

TITLE OF INVENTION

PLANTS AND FEED BAITS FOR CONTROLLING DAMAGE FROM FEEDING INSECTS

APPLICANT(S) FOR DO/EO/US

DAVID DALL

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ☒ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US)
6. ☐ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendment has NOT expired.
   d. ☐ have not been made and will not be made.
8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern other document(s) or information included:

11. ☒ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☒ A FIRST preliminary amendment.
    ☐ A SECOND or SUBSEQUENT preliminary amendment.
14. ☐ A substitute specification.
15. ☐ A change of power of attorney and/or address letter.
16. ☒ Other items or information.
    International Preliminary Examination Report
    International Search Report Prepared by the Australian Patent Office
    Sequency Listing

U.S. APPLIC. NO. (if known, see 37 CFR 1.50)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
09/936216	PCT/AU00/00181	50179-096

	CALCULATIONS	PTO USE ONLY
17. ☒ The following fees are submitted:		
Basic National Fee (37 CFR 1.492(a)(1)-(5)): Search Report has been prepared by the EPO or JPO $860.00		
International preliminary examination fee paid to USPTO (37 CFR 1.482) $690.00		
No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)) $710.00		
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO $1,000.00		
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4) $100.00		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 1,000.00	
Surcharge of $130.00 for furnishing the oath or declaration later than ☐ 20 ☒ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$ 130.00	

Claims	Number Filed	Number Extra	Rate		
Total Claims	22 -20 =	2	x $18.00	$ 36.00	
Independent Claims	3 -3 =	0	x $80.00	$ 0.00	
Multiple dependent claim(s) (if applicable)			+ $270.00	$ 0.00	
			TOTAL OF ABOVE CALCULATIONS =	$ 1,166.00	
Reduction by 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also be filed. (Note 37 CFR 1.9, 1.27, 1.28).				$ 0.00	
			SUBTOTAL =	$ 1,166.00	
Processing fee of $130.00 for furnishing the English translation later than the ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(f)). +				$ 0.00	
			TOTAL NATIONAL FEE =	$ 1.166.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). $40.00 per property +				$ 0.00	
			TOTAL FEES ENCLOSED =	$ 1,166.00	
			Amount to be: refunded	$	
			charged	$	

a. ☐ A check in the amount of $ _____ to cover the above fees is enclosed.

b. ☒ Please charge my Deposit Account No. 500417 in the amount of $ 1,166.00 to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 500417. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

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SIGNATURE Robert L. Price

Robert L. Price
NAME

No. 22,685
REGISTRATION NUMBER

September 10, 2001
DATE

BASED ON FORM PTO-1390 (Rev. 5-93)

Docket No.: 50179-096 **PATENT**

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of	:	
David J. DALL	:	
Serial No.:	:	Group Art Unit: to be assigned
Filed: on even date	:	Examiner: to be assigned

For: PLANTS AND FEED BAITS FOR CONTROLLING DAMAGE FROM FEEDING INSECTS

PRELIMINARY AMENDMENT

Commissioner for Patents
Washington, DC 20231

Sir:

Prior to examination of the above-referenced application, please amend the application as follows:

IN THE CLAIMS:

Please amend the claims as follows:

3. A plant according to claim 1 which expresses a fusolin protein.

5. A plant according to claim 1 which expresses a fusolin-like protein.

7. A plant according to claim 1 which further expresses an exogenous toxin or other agent that is deleterious to insects.

11 A feed bait composition according to claim 9 wherein the one or more constituent protein(s) is a fusolin protein.

13. A feed bait composition according to claim 9 wherein the one or more constituent protein(s) is a fusolin-like protein.

15. A feed bait composition according to claim 9, wherein the spindle bodies, spindle-like bodies or constituent protein(s) comprise 0.05 to 15.0% (by weight) of the composition.

16. A feed bait composition according to claim 9, further comprising a pheromone(s) or other chemical attractive to insects.

17. A feed bait composition according to claim 9, wherein the agriculturally acceptable carrier is selected from edible substances.

18. A method of controlling or preventing damage caused to plants from feeding insects, said method comprising applying to said plant a feed bait composition according to claim 9 before, after or together with an insecticidal chemical and/or biological agent.

19. A method of controlling or preventing damage caused to a plant according to claim 1 from feeding insects, said method comprising applying to said plant an insecticidal chemical and/or biological agent.

20. A method according to claim 18 wherein the insecticidal chemical is selected from organophosphate compounds.

21. A method according to claim 18 wherein the biological agent is selected from pathogenic bacteria.

22. A method according to claim 18 wherein the biological agent is selected from insect viruses.

REMARKS

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned "**Version with markings to show changes made**".

Respectfully submitted,

MCDERMOTT, WILL & EMERY

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Date: September 10, 2001
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Version with markings to show changes made

IN THE CLAIMS:

Claim 3 has been amended as follows:

3. (Amended) A plant according to claim 1 [or 2] which expresses a fusolin protein.

Claim 5 has been amended as follows:

5. (Amended) A plant according to claim 1 [or 2] which expresses a fusolin-like protein.

Claim 7 has been amended as follows:

7. (Amended) A plant according to claim 1 [any one of the preceding claims] which further expresses an exogenous toxin or other agent that is deleterious to insects.

Claim 11 has been amended as follows:

11. (Amended) A feed bait composition according to claim 9 [or 10], wherein the one or more constituent protein(s) is a fusolin protein.

Claim 13 has been amended as follows:

13. (Amended) A feed bait composition according to claim 9 [or 10], wherein the one or more constituent protein(s) is a fusolin-like protein.

Claim 15 has been amended as follows:

15. (Amended) A feed bait composition according to [any one of claims 9-14] claim 9, wherein the spindle bodies, spindle-like bodies or constituent protein(s) comprise 0.05 to 15.0% (by weight) of the composition.

Claim 16 has been amended as follows:

16. (Amended) A feed bait composition according to [any one of claims 9-15] claim 9, further comprising a pheromone(s) or other chemical attractive to insects.

Claim 17 has been amended as follows:

17. (Amended) A feed bait composition according to [any one of claims 9-16] claim 9, wherein the agriculturally acceptable carrier is selected from edible substances.

Claim 18 has been amended as follows:

18. (Amended) A method of controlling or preventing damage caused to plants from feeding insects, said method comprising applying to said plant a feed bait composition according to [any one of claims 9-17] claim 9 before, after or together with an insecticidal chemical and/or biological agent.

Claim 19 has been amended as follows:

19. (Amended) A method of controlling or preventing damage caused to a plant according to [any one of claims 1-8] claim 1 from feeding insects, said method comprising applying to said plant an insecticidal chemical and/or biological agent.

Claim 20 has been amended as follows:

20. (Amended) A method according to claim 18 [or 19], wherein the insecticidal chemical is selected from organophosphate compounds.

Claim 21 has been amended as follows:

21. (Amended) A method according to claim 18 [or 19], wherein the biological agent is selected from pathogenic bacteria.

Claim 22 has been amended as follows:

22. (Amended) A method according to claim 18 [or 19], wherein the biological agent is selected from insect viruses.

Claims:

1. A plant transformed with at least one polynucleotide molecule comprising a nucleotide sequence(s) encoding one or more constituent protein(s) of spindle bodies (SBs) or spindle-like bodies (SLBs) from an insect virus, said nucleotide sequence(s) being operably linked to a suitable promoter sequence(s), wherein said transformed plant expresses said protein(s) in, at least, plant tissue or tissues susceptible to damage by feeding insects.

2. A plant according to claim 1, wherein the one or more constituent protein(s) is/are selected from fusolins, fusolin-like proteins and ER-specific chaperone BiP proteins.

3. A plant according to claim 1 [or 2] which expresses a fusolin protein.

4. A plant according to claim 3, wherein the fusolin protein is selected from fusolins from *Heliothis armigera* EPV (HaEPV), *Pseudaletia separata* EPV (PsEPV), *Choristoneura biennis* EPV (CbEPV) and *Dermolepida albohirtum* EPV.

5. A plant according to claim 1 [or 2] which expresses a fusolin-like protein.

6. A plant according to claim 5, wherein the fusolin-like protein is selected from fusolin-like proteins from *Autographa californica* (AcMNPV), *Bombyx mori* (BmMNPV), *Choristoneura fumiferana* (CfMNPV), *Lymantria dispar* (LdMNPV), *Orgyia pseudotsugata* NPVs (OpMNPV) and *Xestia c-nigrum GV* (XcGV).

7. A plant according to [claim 1 any one of the preceding claims] which further expresses an exogenous toxin or other agent that is deleterious to insects.

8. A plant according to claim 7, wherein the exogenous toxin is selected from *Bacillus thuringiensis* δ-toxin and insect neurohormones.

claim 9
27

18. A method of controlling or preventing damage caused to plants from feeding insects, said method comprising applying to said plant a feed bait composition according to any one of claims 9-17 before, after or together with an insecticidal chemical and/or biological agent.

claim 1

19. A method of controlling or preventing damage caused to a plant according to any one of claims 1-8 from feeding insects, said method comprising applying to said plant an insecticidal chemical and/or biological agent.

20. A method according to claim 18 or 19, wherein the insecticidal chemical is selected from organophosphate compounds.

21. A method according to claim 18 or 19, wherein the biological agent is selected from pathogenic bacteria.

22. A method according to claim 18 or 19, wherein the biological agent is selected from insect viruses.

SEQUENCE LISTING

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        195                 200                 205
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7/pts

PLANTS AND FEED BAITS FOR CONTROLLING DAMAGE FROM FEEDING INSECTS

Field of the Invention:

The present invention relates to the problem of damage caused to plants (e.g. crop plants) from feeding insects such as lepidopterans and coleopterans. More particularly, the present invention relates to a plant capable of expressing, in a tissue or tissues susceptible to damage by feeding insects, an exogenous protein(s) which may reduce damage to the plant by inhibiting feeding, growth and/or development of insects.

Background of the Invention:

Entomopoxviruses (EPVs) are insect-specific members of the family *Poxviridae* (Murphy *et al.*, 1995) that collectively infect hosts such as caterpillars, beetles and locusts (Arif, 1995). Like other members of the poxvirus family (i.e., the chordopoxviruses; ChPVs), EPVs have large double-stranded DNA genomes, produce complex virions, and replicate in the cytoplasm of infected cells (Moss, 1996). While these and other molecular characteristics confirm their poxvirus affinities (Osborne *et al.*, 1996), other notable traits differentiate EPVs from ChPVs, and ally them instead with unrelated groups of insect-infecting viruses. Foremost among these traits is production of the distinctive proteinaceous structures known as spheroids and spindle bodies.

Spheroids develop in the cytoplasm of EPV-infected cells at the site of viral morphogenesis, and when mature, occlude large numbers of infectious virions (Goodwin *et al.*, 1991). They are the agent of horizontal transmission of EPVs, and while their major constituent matrix protein (spheroidin; Hall & Moyer, 1991) has no known homologue outside the taxon, the bodies themselves are assumed to protect virions from detrimental environmental factors such as desiccation and exposure to u.v. light. In this respect they are functionally analogous to the polyhedral bodies which occlude virions of members of the baculovirus family and the cytoplasmic polyhedrosis group of reoviruses.

Most EPVs also encode and produce a protein known as fusolin, which has been shown to be the major constituent of structures known as spindle

bodies (SBs; Dall *et al.*, 1993); these structures have been described from many, but not all, members of EPV genera A and B that infect caterpillars and beetle larvae (Goodwin *et al.*, 1991). In the *Heliothis armigera* EPV (HaEPV)(Fernon *et al.*, 1995), the fusolin protein has a calculated M_r of 40132, and the mature form of the protein has an apparent size of 50K when analysed by SDS-PAGE (Dall *et al.*, 1993). The protein has been found to accumulate in vesicular structures derived from cellular endoplasmic reticulum, where it eventually aggregates and crystallises into SBs (Lai-Fook and Dall, in press). Although other proteins are known to be co-located in SBs (e.g., the ER-specific chaperone protein, BiP; Lai-Fook and Dall, in press), analysis of purified SB preparations shows that fusolin, in its monomeric and multimeric forms (Dall *et al.*, 1993), is by far the most abundant constituent.

Genes encoding homologues of the fusolin protein, in this context known variously as "gp37", "37K protein", "SLP" (spindle-like protein), etc., have also been described from a number of nuclear polyhedrosis (NPV) baculoviruses, including the *Autographa californica*, *Bombyx mori*, *Choristoneura fumiferana*, *Lymantria dispar*, *Orgyia pseudotsugata* NPVs and *Xestia c-nigrum GV* (AcMNPV, BmMNPV, CfMNPV, LdMNPV, OpMNPV and XcGV, respectively; Ayres *et al.*, 1994; Gomi *et al.*, 1999; Liu and Carstens, 1996; Kuzio *et al.*, 1999; Ahrens *et al.*, 1997; Hayakawa *et al.*, 1999). In some of these (e.g., OpNPV; Gross *et al.*, 1993), the protein has been observed within spindle-like bodies (SLBs) in the cytoplasm of infected cells. SLBs have also been observed in the cytoplasm of cells infected with other NPVs (e.g., from *Cadra cautella* NPV, Adams and Wilcox 1968; see also Adams and McClintock, 1991; Cunningham, 1971; Huger and Kreig, 1968; Smirnoff, 1970).

All members of the fusolin group of proteins, irrespective of their viral family of origin, are united by an absolute conservation of amino acid residues at a number of positions in their sequences, in particular in the N-terminal and central regions of the molecule. These conserved residues include HGX (standard one letter amino acid code, where X is an aromatic amino acid) and ARQ motifs near the N-terminal of the deduced protein sequence (Table 1), and e.g. a VRWQR (SEQ ID NO:1) sequence elsewhere within the deduced amino acid sequence (Figure 1). This conservation of sequence elements, like that of the protein's intracellular location, as previously described, suggests that all members of the group also share a

common role in the cycle of virus infection and replication, perhaps in influencing the relationship of the viruses with their hosts (Sriskantha *et al.*, 1997). Nevertheless, the function(s) of members of this group of proteins, and the SB/SLB structures that they form, remain a topic of on-going investigation.

Studies by Xu and Hukuhara (1992, 1994) suggested that a factor associated with preparations of *Pseudaletia separata* EPV (PsEPV), and subsequently identified as fusolin (Hayakawa *et al.*, 1996), was capable of enhancing the infectivity of a heterologous nuclear polyhedrosis virus (*P. unipunctata* NPV). Further studies have shown that a similar effect can be seen in transgenic rice plants in which this protein has been expressed (Hukuhara *et al.*, 1999). Similarly, the SBs of the cupreous chafer (*Anomala cuprea*) have been shown to be capable of acting in the same manner (Mitsuhashi *et al.*, 1998). The role(s) of fusolin protein in the context of homologous EPV systems has not, however, been previously subjected to detailed investigation.

Through experiments involving bioassays using SBs of *Heliothis armigera* EPV (HaEPV) and *Dermolepida albohirtum* EPV ($DaEPV_{SR}$), the present applicants have determined, unexpectedly, that consumption of spindle bodies alone can effect feeding, growth and development of insect larvae. Further, through experiments conducted using recombinant EPVs wherein the fusolin gene has been replaced with a β-galactosidase marker (i.e., to render the recombinant EPVs fusolin negative [$fus^{(-)}$]), the present applicants have also been able to provide evidence to show that it is the fusolin protein component of SBs that is responsible for these effects. Moreover, the latter experiments have indicated that fusolin enhances the infectivity of the homologous EPV virus. As a result, it has been realised that SBs, SLBs and constituent proteins of these structures may be advantageously used in strategies designed to reduce damage caused to plants by feeding insects.

Summary of the Invention:

In a first aspect, the present invention provides a plant transformed with at least one polynucleotide molecule comprising a nucleotide sequence(s) encoding one or more constituent protein(s) of spindle bodies or spindle-like bodies from an insect virus, operably linked to a suitable

promoter sequence(s), wherein said transformed plant expresses said protein(s) in, at least, plant tissue or tissues susceptible to damage by feeding insects.

In a second aspect, the present invention provides a feed bait composition comprising spindle bodies or spindle-like bodies from an insect virus, or one or more constituent protein(s) of said spindle bodies or spindle-like bodies, together with an agriculturally acceptable carrier.

Detailed disclosure of the Invention:

As mentioned above, the invention provides a plant capable of expressing one or more constituent SB/SLB protein(s) in tissues (e.g. leaf tissue or a product tissue such as fruit tissue) susceptible to damage by feeding insects. Thus, when feeding insects feed on a plant according to the invention, they will ingest, along with plant tissue, the expressed constituent SB/SLB protein(s). Since SBs/SLBs appear to inhibit feeding, growth and/or development of insects and, potentially, increase susceptibility to infection from insect pathogens (and thereby insect death), ingestion of one or more of the constituent SB/SLB protein(s) by feeding insects may reduce further damage to the plant. In addition, it is believed that inhibiting the feeding, growth and/or development of insects also increases the likelihood of insect death resulting from, for example, adverse environmental conditions, predators and chemical and other biological agents (e.g. pathogenic bacteria).

The plant according to the invention may be any plant of agricultural, arboricultural, horticultural or ornamental value that is susceptible to damage by feeding insects. Preferably, the plant is selected from plants of agricultural value such as cereals (e.g. wheat and barley), vegetable plants (e.g. tomato and potato) and fruit trees (e.g. citrus trees and apples). Other preferred plants include tobacco and cotton.

The polynucleotide molecule(s) comprising a nucleotide sequence encoding one or more constituent SB/SLB protein(s) operably linked to a suitable promoter sequence(s), may be any polynucleotide molecule(s) that may be stably segregated and retained in daughter cells. Preferably, the polynucleotide molecule(s) is stably integrated into a non-essential site within the plant genome (as may be achieved by the well known technique of homologous recombination).

Preferred constituent SB/SLB proteins are fusolins, fusolin-like proteins and the ER-specific chaperone BiP proteins and homologues thereof.

Preferred fusolin proteins include those from HaEPV, *Pseudaletia separata* EPV (PsEPV), *Choristoneura biennis* EPV (CbEPV) and *Dermolepida albohirtum* EPV (Stone River isolate; $DaEPV_{SR}$; Dall *et al*, unpublished). Most preferred is the fusolin from HaEPV such as is described in the present applicant's Australian Patent No. 668734, the disclosure of which is to be regarded as incorporated herein by reference.

The term "fusolin-like protein" refers to all insect virus proteins and functional fragments thereof which are capable of inhibiting feeding, growth and/or development in at least one insect species, and which preferably also increases susceptibility in at least one insect species to infection from at least one pathogen virus (e.g. a virus). As such, the term includes all proteins (and functional fragments thereof) from entomopoxviruses (EPVs), nuclear polyhedrosis (NPV) and granulosis (GV) baculoviruses, and all other insect viruses, that demonstrate ≥ 35% amino acid sequence identity (as calculated by the GCG Gap algorithm; Devereux *et al.*, 1984) to the HaEPV fusolin protein and which include the following partial amino acid sequences: HGX (standard one letter amino acid code, where X is an aromatic residue), and ARQ motifs near the N-terminal, and VRWQR (SEQ ID NO:1) elsewhere. Preferred fusolin-like proteins include those from AcMNPV, BmMNPV, CfMNPV, LdMNPV, OpMNPV and XcGV.

Where the plant expresses more than one constituent SB/SLB protein, the plant may be transformed with a single polynucleotide molecule such that the proteins are expressed from single or multicistronic messenger RNA. Alternatively, the proteins might be expressed from two or more polynucleotide molecules co-transformed into the plant.

Where the plant expresses all of the constituent SB/SLB protein(s) of an insect virus, the protein(s) may be present in the plant tissues in the form of SB/SLB structures.

Suitable promoter sequence(s) for the expression of the nucleotide sequence(s) encoding the constituent SB/SLB protein(s), may be selected from any promoter sequence which is functional in plants. Preferred promoter sequences include those from plants, plant viruses and plant viroids. Particularly preferred promoter sequences include the cauliflower

mosaic virus (CaMV 35S) promoter element, and promoter elements from the sub-clover stunt virus (SCSV).

Plants according to the present invention may also express an exogenous toxin or other exogenous agent that is deleterious to insects. For example, the plant may also express a *Bacillus thuringiensis* δ-toxin, an insect neurohormone, or an antisense RNA or ribozyme targeted against an essential cellular function. The heterologous toxin or deleterious agent may be encoded by a nucleotide sequence (operably linked to a suitable promoter sequence) borne on the polynucleotide molecule(s) encoding the one or more constituent SB/SLB protein(s) or may be borne on a further polynucleotide molecule which has been co-transformed into the plant.

Transformation of the plant with the polynucleotide molecule(s) may be achieved by any of the methods well known in the art including *Agrobacterium* transformation and electroporation.

As will be appreciated, the benefits achieved by expressing one or more constituent SB/SLB protein(s) in plants might also be achieved by producing feed baits comprising spindle bodies or spindle-like bodies from an insect virus, or one or more constituent protein(s) of said spindle bodies or spindle-like bodies. Thus, feed bait compositions according to the present invention comprise spindle bodies or spindle-like bodies from an insect virus, or one or more constituent protein(s) of said spindle bodies or spindle-like bodies, together with an agriculturally acceptable carrier.

The feed bait compositions may be in a liquid or gel form, but more preferably are in a solid form. The spindle bodies, spindle-like bodies or constituent SB/SLB protein(s) may comprise 0.05 to 15.0% (by weight) of the composition. In addition to the spindle bodies, spindle-like bodies or constituent SB/SLB protein(s) and the agriculturally acceptable carrier, the feed bait composition may further comprise a pheromone(s) or other chemical attractant to insects. For liquid formulations the agriculturally acceptable carrier may be selected from ingredients such as milled clays or edible carrier substances such as plant materials, molasses or raw sugar, and microorganisms such as yeasts or other fungi, algae or bacteria. For solid feed bait compositions, the agriculturally acceptable carrier may be selected from ground or fragmented plant material and other materials as described above processed to an appropriate form. The solid feed bait compositions may be provided as pellets and applied by casting over an area containing a

plant for which protection against damage by feeding insects is desired. Liquid or gelled feed bait compositions may be applied to a plant by spraying.

The spindle bodies, spindle-like bodies or constituent SB/SLB protein(s) included in the feed bait composition may be isolated from natural sources or, more conveniently, produced recombinantly in, for example, bacteria, yeast, insect or mammalian cell cultures.

Insects having ingested spindle bodies, spindle-like bodies or constituent SB/SLB protein(s) as the result of having fed on a plant or feed bait composition according to the present invention may, as mentioned above, be expected to cause reduced damage to plants either as a result of reduced feeding/growth and/or to have reduced life times as a result of an increased susceptibility to adverse environmental conditions or chemical and biological agents. Accordingly, the present invention further extends to methods where a plant in accordance with the first aspect or a plant to which a feed bait composition in accordance with the second aspect has been applied, is treated with an insecticidal chemical and/or biological agent, and especially one whose activity has been shown to be higher against smaller, as compared to larger, insect larvae. Suitable chemical agents include organophosphate compounds and suitable biological agents include pathogenic bacteria and insect viruses (especially *Bacillus thuringiensis* [Bt] and nuclear polyhedrosis baculoviruses). These agents may be applied by any of the methods well known in the art and, most conveniently, by spraying. Preferably, the chemical or biological agent is applied in the form of a composition comprising an agriculturally acceptable carrier. Where used with a feed bait composition, it is to be understood that the feed bait composition might also be applied to the plant before, after or concurrently with the chemical and/or biological agent.

The terms "comprise", "comprises" and "comprising" as used throughout the specification are intended to refer to the inclusion of a stated component, feature or step or group of components, features or steps with or without the inclusion of a further component, feature or step or group of components, features or steps.

The invention is hereinafter described with reference to the accompanying figures and the following, non-limiting examples.

Brief Description of the accompanying figures:

Figure 1: Provides a comparison of a partial amino acid sequence of the fusolin protein of *Dermolepida albohirtum* entomopoxvirus (Stone River isolate; $DaEPV_{SR}$) with corresponding regions of the same protein from other selected entompoxviruses and baculoviruses. Boxed text shows $DaEPV_{SR}$ fusolin sequence as determined by N-terminal amino acid analysis (bold) or conceptual translation of coding nucleotide sequence. Asterisks above the boxed $DaEPV_{SR}$ sequence show residues that differ from others of beetle-derived EPVs (MmEPV and AcEPV); those below the alignment show residues conserved across conceptual proteins from EPVs, NPVs and Gvs. (MmEPV: *Melolontha melolontha* EPV; AcEPV: *Anomala cuprea* EPV; CbEPV: *Choristoneura biennnis* EPV; HaEPV: *Heliothis armigera* EPV; BmNPV: *Bombyx mori* nuclear polyhedrosis virus [NPV]; CfNPV: *Choristoneura fumiferanae* NPV; XcGV: *Xestia c-nigrum* granulosis virus).

Figure 2: Provides a reproduction of a Coomassie blue stained SDS-PAGE gel of fractionated spindle bodies from HaEPV and $DaEPV_{SR}$.

Figure 3: Provides a map for the transfer vector pEPAS3.

Figure 4: Shows protein constituents of wild-type and recombinant [$fus^{(-)}$] isolates of HaEPV, visualised (a) by staining with Coomassie Blue, or (b) by Western blotting with antiserum to HaEPV fusolin. Arrows indicate positions of fusolin protein.

Figure 5: Infectivity of wild-type and recombinant [$fus^{(-)}$] isolates of HaEPV for 48 hr old *Helicoverpa armigera* larvae.

Figure 6: Shows weight gain profiles of 48 hr old *Helicoverpa armigera* larvae after 7 days feeding on diet contaminated with wild-type and recombinant [$fus^{(-)}$] isolates of HaEPV.

Figure 7: Shows the developmental fate of 48 hr old *Helicoverpa armigera* larvae after 21 days feeding on diet contaminated with wild-type and recombinant [$fus^{(-)}$] isolates of HaEPV.

Example 1:

Separation techniques for purification/isolation of HaEPV and $DaEPV_{SR}$ viruses and spindle bodies.

Preparations of spheroids and spindle bodies (SBs) of *Heliothis armigera* entomopoxvirus (HaEPV) and *Dermolepida albohirtum* entomopoxvirus ($DaEPV_{SR}$) were made from macerated cadavers of larvae of *Spodoptera litura* (Lep: Noctuidae) and *Dermolepida albohirtum* (Col: Melolonthinae), respectively, using a process of repeated differential centrifugation. These preparations were layered onto a 36% (w/w) solution of CsCl and spun overnight at 27000 rpm in a Beckman SW41 rotor. Fractions containing high numbers of spindle bodies were collected and pooled, and the process was repeated until a sufficient degree of purity was obtained. Purified preparations of SBs were washed three times in phosphate-buffered saline (PBS), then stored at 5°C in the same solution until use.

Preparations were analysed by light microscopy (LM) and by examination of protein composition by SDS-PAGE, the latter using techniques previously described (Dall *et al.*, 1993). These protocols showed that a very high level of purity could be achieved for HaEPV (Figure 2, lane 2), and that a satisfactory degree of purity could be obtained for $DaEPV_{SR}$ (Figure 2, lane 4).

Partial characterisation of $DaEPV_{SR}$ fusolin

Protein constituents of preparations of $DaEPV_{SR}$ were separated by SDS-PAGE and immobilised by western blotting onto PVDF membrane (Dall *et al.*, 1993). A band corresponding to a protein of about 50K M_r, and thus representing the putative $DaEPV_{SR}$ fusolin protein, was isolated, and the N-terminal amino acid sequence of the immobilised protein was obtained by use of an Applied Biosystems Procise Sequencer.

The resultant amino acid sequence (HGYITFPIARQRR (SEQ ID NO: 2); standard one letter code) was compared with others in GenBank using the NCBI Blast algorithm (Altschul *et al.*, 1990). This and other analyses (using the GCG Gap algorithm) showed that this sequence corresponded to those known from fusolin/gp37 proteins from baculoviruses (nuclear polyhedrosis and granulosis viruses; NPVs and GVs, respectively), and other EPVs, and that it most closely matched forms of the protein previously identified from

EPV isolates from coleopteran hosts (viz., *Melolontha melolontha* EPV [GenBank accession X77616], with which it was identical, and *Anomala cuprea* EPV [AB000780]; Table 1).

Table 1: Alignment of the N-terminal amino acid sequence of $DaEPV_{SR}$ fusolin with other selected sequences.

Virus	Sequence	Sequence Listing No.	GenBank Accession
Dermolepida albohirtum EPV (Stone River isolate)	HGYITFPIARQRR	SEQ ID NO: 2	
Melolontha melolontha EPV	HGYITFPIARQRR	SEQ ID NO: 2	(X77616)
Anomala cuprea EPV	HGYVTFPIARQRR	SEQ ID NO: 3	(AB000780)
Choristoneura biennis EPV	HGYMTFPIARQRR	SEQ ID NO: 4	(M34140)
Heliothis armigera EPV	HGYMTFPIARQRR	SEQ ID NO: 4	(L08077)
Pseudaletia separata EPV	HGYMTFPIARQRR	SEQ ID NO: 4	(BAA09138)
Bombyx mori NPV	HGYLSLPTARQYK	SEQ ID NO: 5	(U55071)
Choristoneura fumiferana NPV	HGYLSVPVARQYK	SEQ ID NO: 6	(U26734)
Mamestra brassica NPV	HGYLSYPVARQYK	SEQ ID NO: 7	(AF108960)
Xestia c-nigrum GV	HGFMLYPLARQYR	SEQ ID NO: 8	(AF162221)
conserved residues	**....*.***..		

Genomic DNA of $DaEPV_{SR}$ was prepared by dissolution of purified preparations of spheroids/SBs in a high pH carbonate buffer containing 40mM thioglycollic acid. After the dissolution of spheroids/SBs was essentially complete (as assessed by LM examination), the solution was neutralised by addition of 10 mM Tris buffer, pH8.0, digested with protease K for 3hr, boiled for 10 minutes then centrifuged at 15K g for 10 minutes to remove residual debris. The viral genomic DNA was collected with the supernatant and stored at -20°C. Viral genomic DNA was used as template in polymerase chain reaction (PCR) protocols with custom oligonucleotide primers (oligos).

A segment of the $DaEPV_{SR}$ fusolin-encoding gene was amplified by use of custom oligos NFUS1 and EPSP6.

Oligo NFUS1 was designed by reverse translation of the $DaEPV_{SR}$ N-terminal amino acid sequence described above, and comprised the sequence:

(NFUS1) 5'-cay ggw tat atr can ttt cct ata gc-3' (SEQ ID NO: 9),

where n represents any nucleotide, r = a or g, w = a or t, and y = c or t.

Oligo EPSP6 was designed to bind to a region known to be highly conserved in other forms of the gene, located some 700 nucleotides downstream of the translation initiation codon, and comprised the sequence:

(EPSP6) 5'-aca rtt rta raa wcc ttc wcc yac-3' (SEQ ID NO: 10),

where n represents any nucleotide, r = a or g, w = a or t, and y = c or t.

PCR amplifications using oligo pair NFUS1 and EPSP6 and $DaEPV_{SR}$ DNA gave rise to a product of approximately 700 bp, as assessed by agarose gel electrophoresis. This product was cloned into plasmid pGem-TEasy (Promega), in order to allow characterisation of its constituent nucleotide sequence. The plasmid was replicated in *Escherichia coli* strain DH10β, and purified with a commercial reagent/protocol (Wizard Prep; Promega).

Analysis of the amplified nucleotide sequence used universal forward and reverse oligo nucleotides, with Elmer Perkin "Big Dye" reaction mix and PCR cycle sequence methodology as recommended by that supplier. Products of the sequencing reaction were analysed on an ABA377 DNA sequencer. The DNA sequence obtained was analysed using the GCG Map and Translate algorithms (Devereux *et al.*, 1984); related sequences were obtained from GenBank using the NCBI Blast algorithm.

Comparative sequence analyses used GCG Gap and PileUp algorithms (Devereux et al., 1984). As shown in Table 1, the available $DaEPV_{SR}$ fusolin amino acid sequence shows closest relationships to analogous regions (as construed by alignment from the N-termini of the mature forms) of fusolin proteins from EPVs of coleopteran hosts (MmEPV and AcEPV), but also shows significant levels of sequence identity to other fusolin and gp37 proteins from EPVs and baculoviruses, respectively, from lepidopteran hosts.

Alignment of conceptual amino acid sequences (Figure 1) shows that $DaEPV_{SR}$ fusolin has a unique sequence (as indicated by asterisks above the line, which show positions that differ with respect to other sequences of coleopteran EPV origin), but also that it retains the same groupings of conserved residues found in related proteins from other EPVs and baculoviruses from coleopteran and lepidopteran origins (asterisks below the alignment). As shown in Table 2, percentage identities between selected fusolin sequences range from 38.0 to 89.7% for HaEPV (complete molecule) and 45.2 to 81.8 % for $DaEPV_{SR}$ (corresponding regions).

Table 2: Relationships of deduced HaEPV and DaEPV$_{SR}$ fusolin proteins with homologues from other entomopox- and baculovirus sources.

		% identity	% similarity	
[a]HaEPV	/ PsEPV		89.7	92.9
	/ CbEPV		62.9	72.6
	/ AcEPV		55.5	71.3
	/ MmEPV		52.3	67.6
	/ MbNPV		46.3	61.2
	/ BmNPV		42.8	61.6
	/ XcGV		41.7	63.6
	/ CfNPV		38.0	58.8
[b]DaEPV$_{SR}$	/ MmEPV	(19-238)	81.8	87.3
	/ AcEPV	(17-236)	72.7	81.4
	/ CbEPV	(21-241)	60.5	75.0
	/ HaEPV	(21-240)	63.6	78.2
	/ BmNPV	(20-236)	47.7	62.0
	/ CfNPV	(20-236)	45.2	61.8
	/ XcGV	(19-225)	47.0	66.8

Identities and GenBank accession numbers of viruses/sequences shown in Table 2 are as presented in Table 1.

[a] Comparison of HaEPV fusolin with other viral homologues employs deduced full length protein sequences, using the GCG Gap algorithm at default gap weight and penalty settings.

[b] Comparison of DaEPV$_{SR}$ fusolin with other homologues employs the deduced partial DaEPV sequence as presented in Figure 1, and the corresponding regions of other fusolin homologues, also as shown in Figure 1. Those regions of the latter proteins are identified in Table 2 (in brackets) by their amino acid residue numbers in their respective full length sequences. Comparisons used the GCG Gap algorithm as described above.

Bioassay of spindle body constituents against caterpillars

Purified SBs of HaEPV and DaEPV$_{SR}$ were incorporated into artificial insect diet by addition and mixing when the preparation was at a temperature just above solidification point. Diet was then allowed to solidify, and was administered to neonate larvae of *Helicoverpa armigera* and *Spodoptera litura*. Larvae were individually housed, and were reared in darkness at a constant temperature of 28°C. Larval weights and developmental status were assessed periodically; resultant pupae were stored at 5°C prior to examination (see below).

Care was taken to exclude any contribution of contaminant virus infection to outcomes of experiments. In the case of experiments using HaEPV, weights and development times of individual larvae were included in analyses only when (1) the individual larva successfully pupated and showed a normal pupal morphology, and (2) the resultant pupa was judged not to be infected by virus, as assessed by examination of tissue by light microscopy. Thus, in these experiments every individual pupa was examined before inclusion of associated data into analyses. In the case of experiments using $DaEPV_{SR}$, previous work has shown that neither caterpillar species used in bioassays here is susceptible to infection with this beetle-derived pathogen. Nevertheless, as above, data were only included in analyses in cases where individual larvae successfully pupated and showed a normal pupal morphology; in these experiments however, only pupae from larvae exposed to the highest dosage of $DaEPV_{SR}$ spindle bodies in any given experiment were assessed for the presence of virus. This methodology was designed to firstly ensure the validity of earlier studies, as noted above, and, secondly, to preclude the possibility of accidental contamination of larvae or experimental inoculum with viruses from other sources. No instance of $DaEPV_{SR}$ replication was observed.

Experiment A (#05-90526)

The experiment aimed to determine whether consumption of EPV SBs and associated fusolin protein would result in reduced rates of growth of *Helicoverpa armigera* caterpillars. Accordingly, neonate larvae of *H. armigera* were exposed to three dose rates of fusolin in SBs of HaEPV and $DaEPV_{SR}$ (dosages of 5, 50 and 100 µg fusolin/cc diet), and were assessed as described above. Seven days after commencement of the experiment, weights of subsequently "qualifying" larvae (see above) were as shown in Table 3 below:

Table 3: Weights of *Helicoverpa armigera* larvae after consumption of diet containing EPV spindle body constituents for seven days.

treatment	dose (μg/cc diet)	sample size	mean wt (gm)	standard error
control	none	23	0.0714	0.0097
HaEPV	5	27	0.0521	0.0083
HaEPV	50	21	0.0543	0.0094
HaEPV	100	16	0.0233	0.0107
$DaEPV_{SR}$	5	45	0.0642	0.0071
$DaEPV_{SR}$	50	48	0.0525	0.0069
$DaEPV_{SR}$	100	44	0.0591	0.0072

Examination of data by analysis of variance (ANOVA) showed no difference between mean weights of larvae in the control group and those fed preparations of $DaEPV_{SR}$ SBs ($P = 0.2285$), but showed that after seven days' exposure, larvae fed HaEPV SBs were significantly smaller than those in the control group ($P = 0.0201$). Analysis of larval response to different dosages of HaEPV SBs failed ($P = 0.0632$) to show evidence of a significant relationship at a 5% confidence level.

These data indicate that short term exposure of *H. armigera* larvae to the constituents of HaEPV SBs can lead to significant reductions in growth of the animal.

Experiment B (#07-90630)

The experiment aimed to determine whether consumption of $DaEPV_{SR}$ SBs would affect growth of *Helicoverpa armigera* larvae if continued for a more lengthy period, or whether consumption for an initial seven day period (as previously tested in Experiment A) would have an observable effect after a longer period of development. Accordingly, neonate larvae of *Helicoverpa armigera* were exposed to three dosages of $DaEPV_{SR}$ fusolin in SBs, and then assessed as described above. After seven days of feeding, larvae were weighed, and for each dosage regime, one sub-group was then allowed to feed on normal diet ("7d exp/ 7d normal"), while the other continued to feed on a diet containing SBs ("14d exp"). After 7 days, no significant difference was observed between mean weights of control larvae and those exposed to

DaEPV$_{SR}$ SBs (P= 0.7791); this result is consistent with that reported from Experiment A above. After 14 days, all larvae were reweighed, with results as shown in Table 4 below.

Table 4: Weights of *Helicoverpa armigera* larvae after consumption of diet containing EPV spindle body constituents for seven days (with subsequent seven days feeding on regular diet), or continuously for 14 days.

treatment	fusolin dose (µg/cc diet)	sample size	mean wt (gm)	standard error
control	none	9	0.4605	0.0389
7d exp/ 7d normal	5	16	0.4313	0.0348
14d exp	5	11	0.2194	0.0176
7d exp/ 7d normal	50	17	0.4325	0.0337
14d exp	50	10	0.2812	0.0185
7d exp/ 7d normal	100	15	0.3850	0.0359
14d exp	100	9	0.2821	0.0195

Examination of data by analysis of variance (ANOVA) showed no difference between the mean weights of larvae in the control group and those fed preparations of DaEPV$_{SR}$ SBs for 7 days prior to subsequent feeding for a further 7 days on normal diet (P = 0.3857). In contrast, highly significant differences were apparent between the mean weights of larvae in the control group and those continuously fed preparations of DaEPV$_{SR}$ SBs for 14 days (P = 0.0000), and between mean weights of the "7 day exposure/7 day normal diet" and "14 day continuous exposure" groups (P= 0.0000).

This experiment thus indicates that short term exposure of *H. armigera* larvae to the constituents of DaEPV$_{SR}$ SBs has neither short-term nor longer-term consequences, but that continuous exposure for longer periods (e.g. 14 days) causes highly significant reduction in growth. Taken across all DaEPV$_{SR}$ dosages, mean caterpillar weight was 0.2588 gm after 14 days exposure, as compared to a mean weight of 0.4605 gm for unexposed animals, representing a reduction in growth of 44%.

Experiment C (#14-91007)

The purpose of this experiment was to determine whether consumption of either HaEPV or $DaEPV_{SR}$ SBs and associated fusolin protein would affect growth of *Spodoptera litura* caterpillars, either after a 7 day period of exposure, a 7 day exposure followed by 5 days' access to normal diet, or continuous exposure for a 12 day period. Accordingly, neonate larvae of *S. litura* were exposed to one dose of SBs and associated fusolin of HaEPV (5 µg fusolin/cc diet), and to two dose rates of $DaEPV_{SR}$ SBs and associated fusolin (5 and 50 µg fusolin/cc diet).

After seven days of feeding larvae were weighed, and for each dosage regime, one sub-group was then allowed to feed on normal diet ("7d exp/5d normal"), while the other continued to feed on diet containing SBs ("12d exp"). After 7 days' feeding activity, no significant differences were observed between mean weights of control larvae and those exposed to various dosages of SBs/fusolin. After a total of 12 days feeding, larvae were reweighed, with results as shown in Table 5 below.

Table 5: Weights of *Spodoptera litura* larvae after consumption of diet containing EPV spindle body constituents for seven days (with subsequent five days feeding on regular diet), or continuously for 12 days.

treatment	fusolin identity and dose (µg/cc diet)	sample size	mean wt (gm)	standard error
control	none	25	0.7213	0.0464
7d exp/ 5d normal	HaEPV; 5	14	0.7174	0.0557
12d exp	HaEPV; 5	11	0.1719	0.0629
7d exp/ 5d normal	$DaEPV_{SR}$; 5	21	0.6829	0.0509
12d exp	$DaEPV_{SR}$; 5	17	0.1750	0.0566
7d exp/ 5d normal	$DaEPV_{SR}$; 50	16	0.5415	0.0509
12d exp	$DaEPV_{SR}$; 50	15	0.1520	0.0526

Examination of data by analysis of variance (ANOVA) showed no difference between the mean weights of larvae in the control group and those fed preparations of either HaEPV or $DaEPV_{SR}$ SBs for 7 days prior to subsequent feeding for a further 5 days on normal diet (P = 0.2973). In contrast, highly significant differences were apparent between the mean weights of larvae in the control group and those continuously fed preparations of HaEPV SBs for 12 days (P = 0.0000), or $DaEPV_{SR}$ SBs for 12 days (P = 0.0000). Likewise, highly significant differences were apparent between the mean weights of larvae in the group fed HaEPV SBs for seven days only before feeding for 5 days on uncontaminated diet, and those continuously fed preparations of HaEPV SBs for 12 days (P = 0.0000). Similarly, weights for the same comparison at each dose rate of $DaEPV_{SR}$ SBs were highly significant (P = 0.0000 for both dose rates).

These data indicate that short term (i.e., up to 7 days) exposure of *S. litura* larvae to the constituents of HaEPV and $DaEPV_{SR}$ SBs has neither short-term nor longer-term consequences, but that continuous exposure for longer periods (e.g. 12 days) causes highly significant reduction in growth.

Example 2:

Preparation of fusolin negative recombinant EPV

In this example, the transfer vector pEPAS3 (Figure 3), which contains a bacterial *lacZ* gene inserted immediately upstream of the HaEPV fusolin coding sequence, in a manner that prevents expression of the latter, was used, together with wild-type HaEPV, to produce recombinant forms of HaEPV in which fusolin production was replaced by production of the β-galactosidase marker protein. Amplified stocks of that recombinant HaEPV were subsequently found to contain forms of the virus which produced neither the β-galactosidase marker nor the fusolin protein, as judged by the absence of SBs in preparations viewed by light microscopy. Two such variants (pp5 and pp7) were isolated by repeated plaque purification and subsequent re-amplification in insect cell cultures. Harvested preparations of cells infected with these viruses were then fed to larvae of the moth *Helicoverpa armigera*, establishing, in turn, infections in those insects. Infected insects were processed to recover the products of these infections for use in subsequent biological investigations, and preparations of virus stocks

known as pp7T6 and pp7S22 (or, following a second insect passage, pp7T6/5 and pp7S22/13) were ultimately selected for more detailed characterisation.

Stocks of the wild-type clonal isolate wt#2/011293 (Osborne *et al.*, 1996), which was used as the parental form for production of the original β-galactosidase expressing recombinant, were carried in parallel through plaque purification, re-amplification, and feeding to recovery from *H. armigera* insect hosts. Selected lines from these stocks (2C1 and 2D8, or 2C1/11 and 2D8/17) served as controls in the investigations described below.

Light microscopy and scanning electron microscopy was used to examine the composition and morphology of preparations of stocks pp7T6, pp7S22, 2C1 and 2D8. As expected, preparations of the wild-type viruses 2C1 and 2D8 were observed to contain both virus spheroids and SBs, while preparations of the recombinants pp7T6 and pp7S22 were observed to contain only spheroids. The spheroids of all four stocks appeared to be morphologically identical.

The molecular composition of preparations of these virus stocks was examined using the standard laboratory protocols of SDS-PAGE and Western blotting (see, for example, Sambrook *et al.*, 1989). As shown in Figure 4(a), Coomassie Blue staining of the separated protein constituents of all four preparations showed a prominent band of about 115 kDa, corresponding to the major spheroid matrix protein (spheroidin; Hall & Moyer, 1991; Sriskantha *et al.*, 1997), and numerous other less intense bands apparently common to each. Preparations of the two wild-type stocks also showed a band of protein with a mobility of about 50 kDa, (Figure 4[a], arrow) corresponding to the monomeric form of the fusolin protein (Dall *et al.*, 1993), that was not apparent in preparations of pp7T6 and pp7S22. A polyclonal antiserum to HaEPV fusolin protein (Dall *et al.*, 1993) and Western blotting protocols were then used to further characterise these virus stocks. As shown in Figure 4(b), both preparations of wild-type virus produced very prominent immuno-reactive bands at a position corresponding to a molecular weight of about 50kDa (arrow), which, as expected, were not apparent in preparations of the two fusolin negative [$fus^{(-)}$] recombinant forms.

Feeding studies with fusolin negative recombinant EPV

One wild-type isolate (2D8) and one recombinant (pp7T6) were then selected for more detailed biological characterisation. Individually housed 48 hour old *H. armigera* larvae were exposed to a range of quantities of each

of the viruses by placing them on artificial diet spread with aliquots of virus dilution series. Seven days later ("post-infection"; 7 dpi) each larvae was weighed and at 21 dpi all larvae were collected, their developmental stage was recorded, and their status with respect to viral infection (i.e. infected or uninfected) was determined by examination of fat body smears by light microscopy. In all instances, larvae that died at or before 7 dpi were excluded from the assay, while those that were dead at 21 dpi were considered to be positive (i.e. infected).

As shown in Figure 5, these experiments demonstrated that the wild-type virus isolate 2D8 was substantially more infectious than the $fus^{(-)}$ recombinant pp7T6, with the former having an estimated IC_{50} (this being the quantity of virus required to infect 50% of exposed larvae) of 0.2 spheroids/mm^2 diet (sph/mm^2), while for pp7T6 it was 35 sph/mm^2. Results of less detailed investigations with virus isolates 2C1 and pp7S22 were also consistent with these results.

Further analysis of the results has revealed, in addition, another fusolin associated phenomenon which has not previously been recognised, namely, that the presence of fusolin is associated with retardation of the rates of growth and development of exposed insect larvae. Thus, Figure 6 shows mean weights of infected insects only, taken at 7 dpi, and calculated as a proportion of the weight of uninfected larvae from the same cohort (i.e. as a % of the weight of experimental controls). As can be observed, when the results were analysed in this manner it was clear that in the presence of fusolin, larval weight gain was much reduced. This analysis thus makes allowance for the previously described observation (i.e. that the presence of fusolin enhances virus infectivity), and further shows that when intrinsic infectivity of a particular dose is used as the basis of comparison, this previously unrecognised effect of fusolin on insect growth can be observed.

Similarly, and as shown in Figure 7, when the developmental fate of those same infected insects, now pooled in three "categories" of infection rates, was assessed at 21 dpi, a much reduced proportion of larvae was observed to proceed to pupation in samples exposed to preparations of the wild type virus containing the fusolin protein.

The above examples demonstrate the feasibility of strategies designed to effect oral ingestion of constituent SB/SLB protein(s) such as fusolin by feeding insects as a means of inhibiting feeding, growth and/or development of such insects. Such strategies may therefore be of significant value with respect to limiting losses to commodity materials that result from insect feeding activity. That is, it can be appreciated that small insects cause less feeding damage to plants than do larger ones, and that retarding the growth and/or development of insects will increase the time-span during which factors such as adverse environmental conditions, predators, and/or artificially applied chemical and biological agents may effect their control. In addition, it is widely recognised that early instar (i.e., smaller) insects are intrinsically more susceptible to infection with, or the activity of, a variety of chemical and biological control agents such as the bacterium *Bacillus thuringiensis* ("Bt").

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

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Claims:

1. A plant transformed with at least one polynucleotide molecule comprising a nucleotide sequence(s) encoding one or more constituent protein(s) of spindle bodies (SBs) or spindle-like bodies (SLBs) from an insect virus, said nucleotide sequence(s) being operably linked to a suitable promoter sequence(s), wherein said transformed plant expresses said protein(s) in, at least, plant tissue or tissues susceptible to damage by feeding insects.

2. A plant according to claim 1, wherein the one or more constituent protein(s) is/are selected from fusolins, fusolin-like proteins and ER-specific chaperone BiP proteins.

3. A plant according to claim 1 or 2 which expresses a fusolin protein.

4. A plant according to claim 3, wherein the fusolin protein is selected from fusolins from *Heliothis armigera* EPV (HaEPV), *Pseudaletia separata* EPV (PsEPV), *Choristoneura biennis* EPV (CbEPV) and *Dermolepida albohirtum* EPV.

5. A plant according to claim 1 or 2 which expresses a fusolin-like protein.

6. A plant according to claim 5, wherein the fusolin-like protein is selected from fusolin-like proteins from *Autographa californica* (AcMNPV), *Bombyx mori* (BmMNPV), *Choristoneura fumiferana* (CfMNPV), *Lymantria dispar* (LdMNPV), *Orgyia pseudotsugata* NPVs (OpMNPV) and *Xestia c-nigrum GV* (XcGV).

7. A plant according to any one of the preceding claims which further expresses an exogenous toxin or other agent that is deleterious to insects.

8. A plant according to claim 7, wherein the exogenous toxin is selected from *Bacillus thuringiensis* δ-toxin and insect neurohormones.

9. A feed bait composition comprising spindle bodies (SBs) or spindle-like bodies (SLBs) from an insect virus, or one or more constituent protein(s) of said spindle bodies or spindle-like bodies, together with an agriculturally acceptable carrier.

10. A feed bait composition according to claim 9, wherein the one or more constituent protein(s) is/are selected from fusolins, fusolin-like proteins and ER-specific chaperone BiP proteins.

11 A feed bait composition according to claim 9 or 10, wherein the one or more constituent protein(s) is a fusolin protein.

12. A feed bait composition according to claim 11, wherein the fusolin protein is selected from fusolins from *Heliothis armigera* EPV (HaEPV), *Pseudaletia separata* EPV (PsEPV), *Choristoneura biennis* EPV (CbEPV) and *Dermolepida albohirtum* EPV.

13. A feed bait composition according to claim 9 or 10, wherein the one or more constituent protein(s) is a fusolin-like protein.

14. A feed bait composition according to claim 13, wherein the fusolin-like protein is selected from fusolin-like proteins from *Autographa californica* (AcMNPV), *Bombyx mori* (BmMNPV), *Choristoneura fumiferana* (CfMNPV), *Lymantria dispar* (LdMNPV), *Orgyia pseudotsugata* NPVs (OpMNPV) and *Xestia c-nigrum GV* (XcGV).

15. A feed bait composition according to any one of claims 9-14, wherein the spindle bodies, spindle-like bodies or constituent protein(s) comprise 0.05 to 15.0% (by weight) of the composition.

16. A feed bait composition according to any one of claims 9-15, further comprising a pheromone(s) or other chemical attractive to insects.

17. A feed bait composition according to any one of claims 9-16, wherein the agriculturally acceptable carrier is selected from edible substances.

18. A method of controlling or preventing damage caused to plants from feeding insects, said method comprising applying to said plant a feed bait composition according to any one of claims 9-17 before, after or together with an insecticidal chemical and/or biological agent.

19. A method of controlling or preventing damage caused to a plant according to any one of claims 1-8 from feeding insects, said method comprising applying to said plant an insecticidal chemical and/or biological agent.

20. A method according to claim 18 or 19, wherein the insecticidal chemical is selected from organophosphate compounds.

21. A method according to claim 18 or 19, wherein the biological agent is selected from pathogenic bacteria.

22. A method according to claim 18 or 19, wherein the biological agent is selected from insect viruses.

09/936216

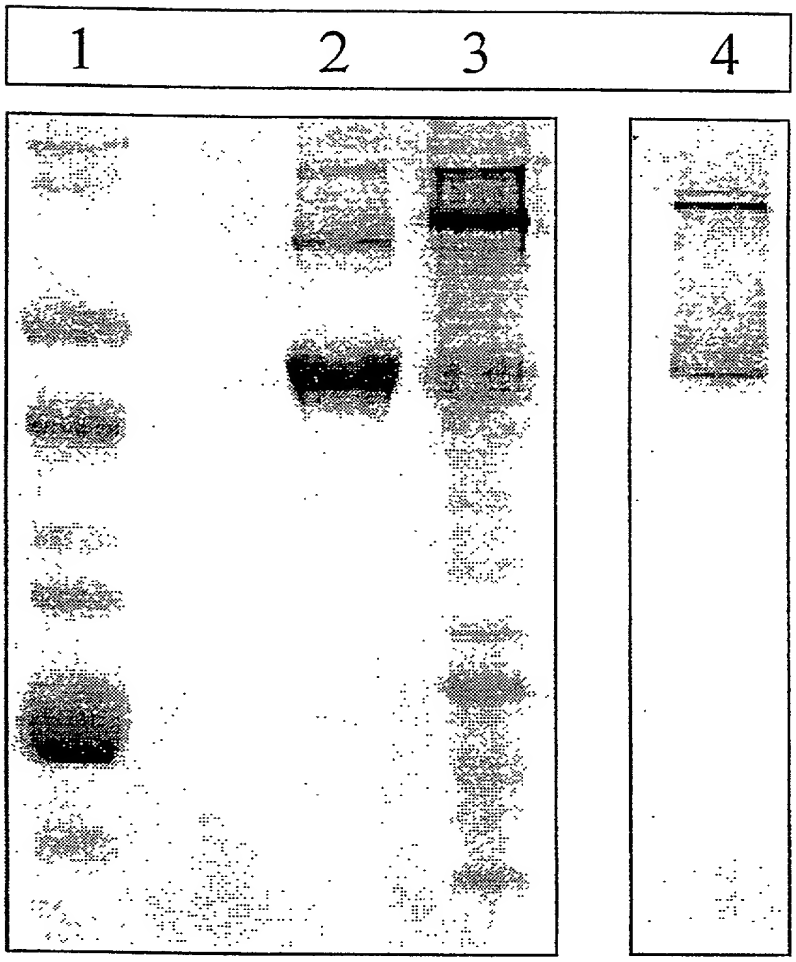
Figure 1

```
                                      **  *           *        **  *                *
DaEPV
HGYITFPIARQRRCNVQGGFWWPTDGSAIPDPMCRAAYQNVFNTVLQQ.GGSLNQAATAAQYMFQQDNEYAALAGSNFRD
MmEPV      HGYITFPIARQRRCNVQGGFWWPPGGSGIPDPMCRAAYQNVYNKVLQQ.GGTIDQAASAAQYMFQQDNEYAALAGPNYLD
AcEPV      HGYVTFPIARQRRCNVQGGFWWPPEGTNIPDPMCRAAYQYVFNKVLSE.GGSTSQAASAAQYMFQQDNEYAALAGPNFRD
CbEPV      HGYMTFPIARQRRCSAAGGNWYPVGGGGIQDPMCRAAYQNVFNKVLNSNGGDVIDASEAANYMYTQDNEYAALAGPDYTN
HaEPV      HGYMTFPIARQRRCSVRGGQWWPPNGDGITDTMCRAAYQNVYNKVLNQY.NDPQEAATAAQYMFQQDNEYAALAGPDYTN
BmNPV      HGYLSLPTARQYKCFKGGNFYWPHNGDKIPDAACRNAYKSVYYK.YRALDLESGAAAATAQYMFQQYMEYASVAGPNYDD
CfNPV      HGYLSVPVARQYKCFRDGNFWWPNNGDNIPDEACRNAYKKVYYK.YRAINVPSQEAASAAQYMFQQYTEYAALAGPNYLD
XcGV       HGFMLYPLARQYRCYAPQDFYWPDDGSNIQNPACKLAFQHVY..........RNSGSAAAQYMFVQYAEYAALAGSNYND
           **    * ***  *       *  *  *    *  *   *                ***** *  **** **

             *   *    **      **  **     *   *     *    *   * *                *
DaEPV
LNHIQNNVVPFDLCAAGANNWRRVPFGDKSGMDISGSWTPTGIPLESNTVGTGPIEFEFCPTAIHEPSFFEIYITVPNFN
MmEPV      QNHIRNNVVPNYLCAAHATTWRIRPFGDKTGMDVSGSWTPTVIPLQDNTVSTVPIEFEFCPTAIHEPSFFEIYITVPSFN
AcEPV      ICWIKEQVVPDYLCAAGADTWRIRPFGDKTGMDIVGSWPPTVIPLENNFVNTIPIELEFCPTAIHEPSYFEVYVTTPEFN
CbEPV      ICHIQQRVVPSYLCAAGASDWSIRPFGDKSGMDLPGSWTPTIIQLSDNQQSNVVMELEFCPTAVHDPSYYEVYITNPSFN
HaEPV      LCNLQQNVVPNNLCAAGADDWDVVPFGDKSGMDLPGNWVPTVIPLDSNHQSSVALELEFCPTAVHDPSYYEVYITNSGFN
BmNPV      FDLIKQRVVPHTLCGAGSNDRNS.VFGDKSGMDEPFNNWKPNTLYLNLYQPVYRMNVHFCPTAIHEPSYFEVFITKSNWD
CfNPV      FDMVKRDVVPHTLCGAASNDRAA.LFGDKSGMDEPFYNWRPDVLYMNRYQNSYPMDVHFCPTAIHEPSYFEVFVTKSTWD
XcGV       MQHIQQDVVPNFLCSAAADNTST.PYGDKSGISLPSDHWQTTII.....NDRGHTQLYYCPTVPHDPSFFQVFVTKKDFD
                *** ** *        *** *                              *** * **      *

             *    *                         *   *    *
DaEPV
VFTDQVTWSQLENIFTGPIPLVARR....PDSLCNANSRVYRITVGIPMRQTQFVLYVRWQRIDP               (SEQ ID NO: 11)
MmEPV      VYTDQVTWQQLINIFTGPIPLVQRR....PDSQCNAHNLVYRTTVGIPVRQTQFVLYVRWQRNDP        (SEQ ID NO: 12)
AcEPV      VYRDKVTWPLLELVFNSTVPLVNRR....ADSLCTANARVYRMIVPVPYRQTQFVIYVRWQRIDP        (SEQ ID NO: 13)
CbEPV      VYTDNVVWANLDLIYNNTVTLRPKL....PESTCAANSMVYRFEVSIPVRPSQFVLYVRWQRIDP        (SEQ ID NO: 14)
HaEPV      VHTDNVVWGNLELIFNDTVPLRPKS....STSTCNANPNVYRFTVSIPVRPAQFVLYVRWQRIDP        (SEQ ID NO: 15)
BmNPV      RRNPI.TWNELEYIGGNDSDLIPNP....GDPLCD.NSLVYSIPVVIPYRSNQFVMYVRWQRIDP        (SEQ ID NO: 16)
CfNPV      RRNPI.TWNELEYIGGNNSGLVPNP....GDPLCD.SNQIYSIPVSVPYRSGQFVMYVRWQRIDP        (SEQ ID NO: 17)
XcGV       VGTTIVTWNDLELVHEQSAVIVPNSRTVPNSEEC. GAFVYSIDATLPMRSKPFVVFVRWQREDP        (SEQ ID NO: 18)
               *  *                 *        *         **  ***** **
```

FIGURE 2



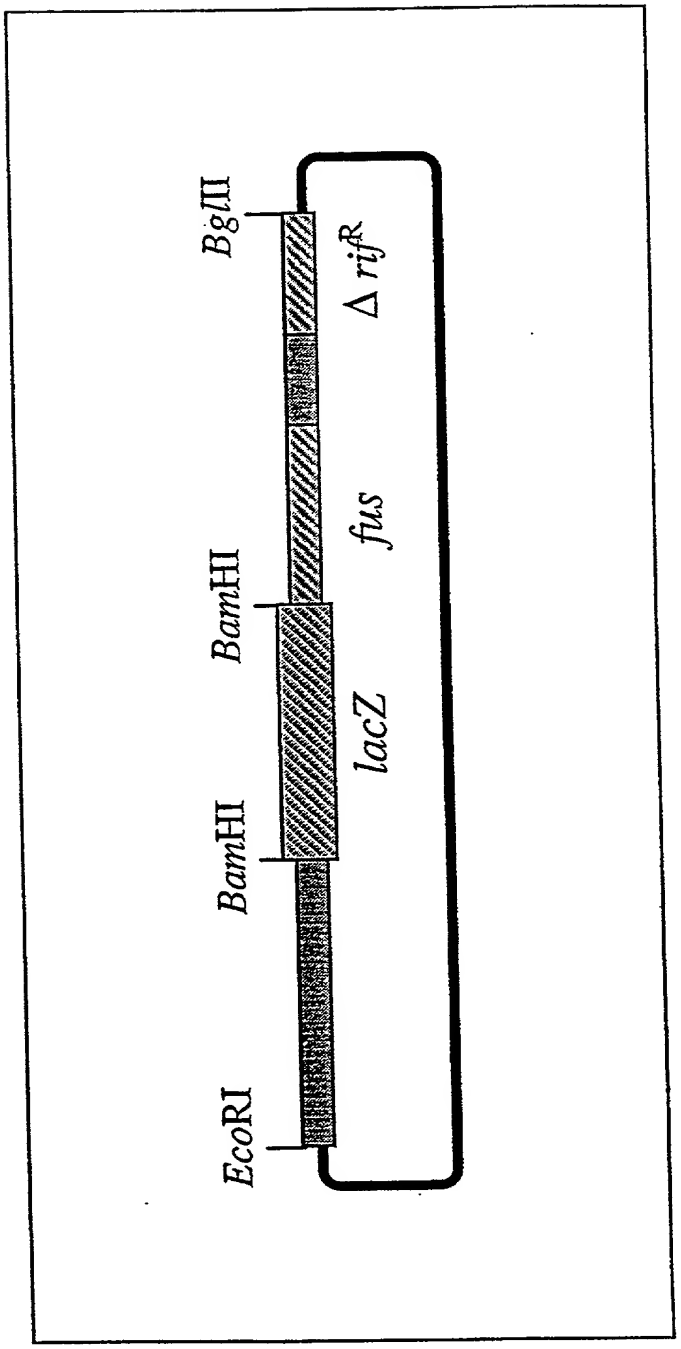


FIGURE 3

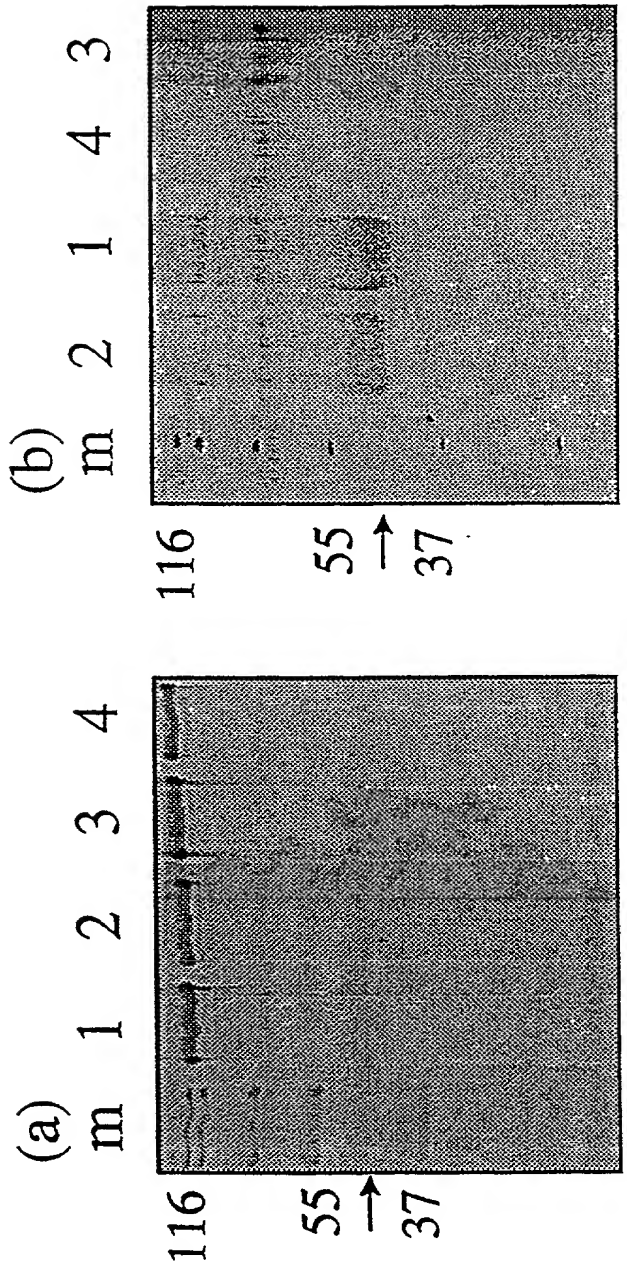


FIGURE 4

FIGURE 5

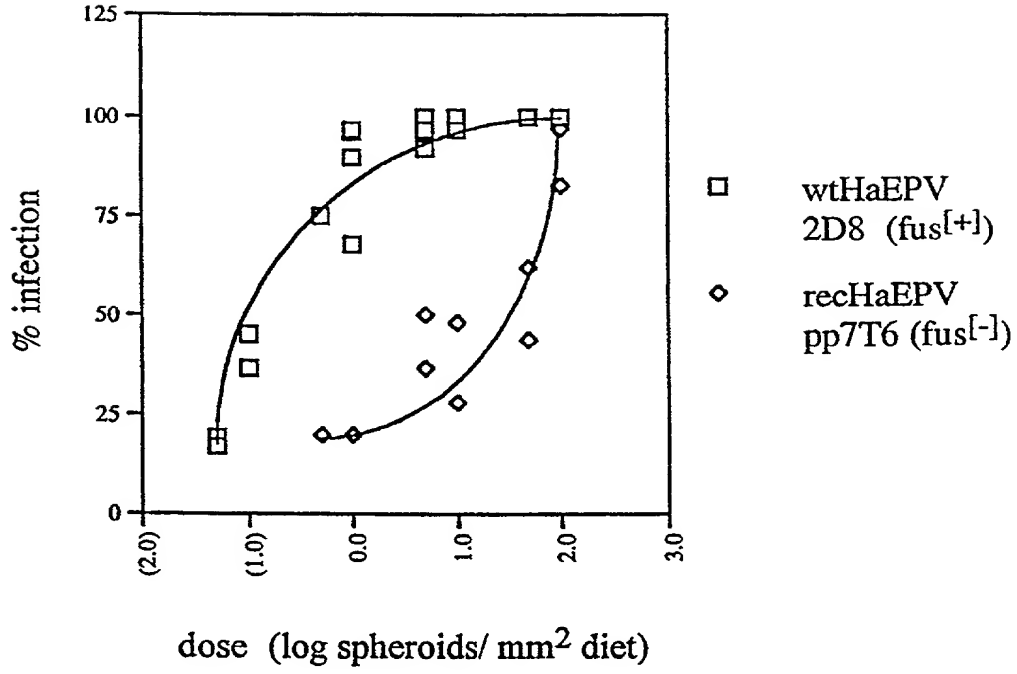

125
100
75
50
25
0
% infection
(2.0)
(1.0)
0.0
1.0
2.0
3.0
dose (log spheroids/ mm2 diet)
wtHaEPV
2D8 (fus[+])
recHaEPV
pp7T6 (fus[-])

FIGURE 6

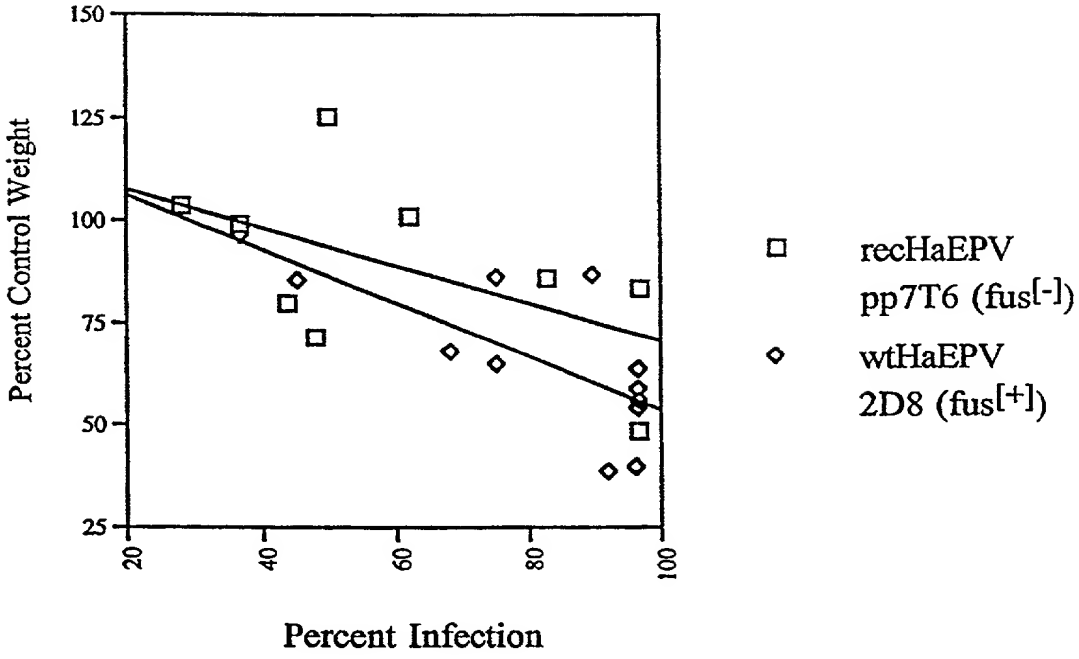
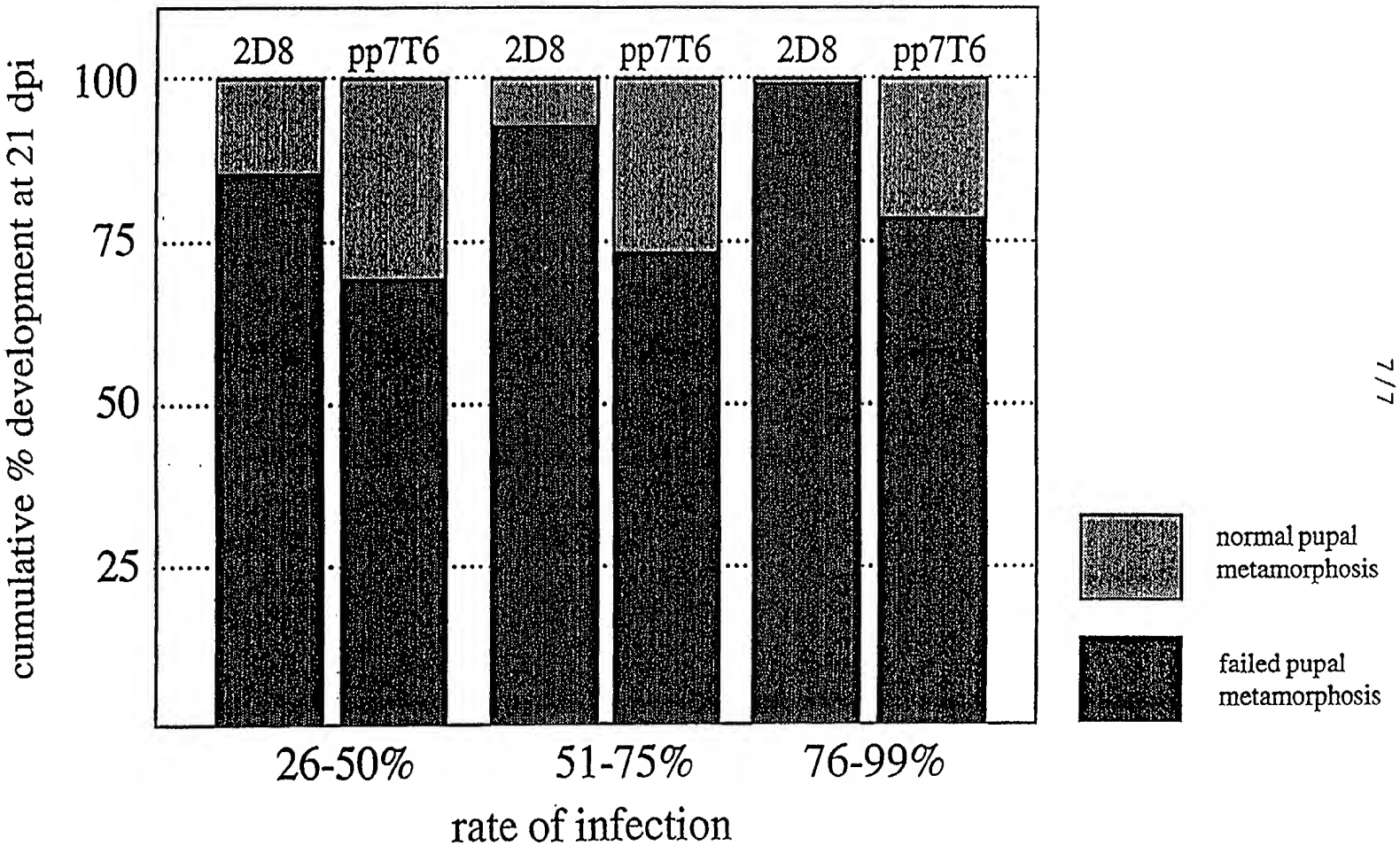

150
125
100
75
50
25
Percent Control Weight
20
40
60
80
100
Percent Infection
recHaEPV
pp7T6 (fus[-])
wtHaEPV
2D8 (fus[+])

FIGURE 7



COMBINED DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY (Includes Reference to PCT International Application(s))	Attorney's Docket Number 50179-096

As below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

PLANTS AND FEED BAITS FOR CONTROLLING DAMAGE FROM FEEDING INSECTS

the specification of which:

☐ is attached hereto.

☒ was filed as United States application Serial No. 09/936,216

on September 10, 2001

and was amended on September 10, 2001 (if applicable).

☒ was filed as PCT international application Number PCT/AU00/00181

on March 20, 2000

and was amended under PCT Article 19 on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is known to me to be material to patentability in accordance with Title 37, Code of Federal Regulations, §1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, §119(a)-(d) or Section 365(b) of any foreign and/or international application(s) for patent or inventor's certificate or Section 365(a) of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed:

PRIOR FOREIGN/PCT APPLICATION(S) AND ANY PRIORITY CLAIMS UNDER 35 U.S.C. 119:

COUNTRY (if PCT, indicate "PCT")	APPLICATION NUMBER	DATE OF FILING (day, month, year)	PRIORITY CLAIMED UNDER 35 USC 119
PCT	PCT/AU00/00181	March 10, 2000	☒Yes ☐ No
AUSTRALIA	PP 9113	March 10, 1999	☒Yes ☐ No

I hereby claim the benefit under 35 USC §119(e) of any United States provisional application(s) listed below.

PRIOR PROVISIONAL APPLICATION(S):

Application Number	Filing Date

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s), or §365(c) of any PCT international application(s) designating the United States of America that is/are listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in that/those prior application(s) in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, §1.56 which occurred between the filing date of the prior application(s) and the national or PCT international filing date of this application.

PRIOR U.S. APPLICATIONS OR PCT INTERNATIONAL APPLICATIONS DESIGNATING THE U.S. FOR BENEFIT UNDER 35 U.S.C. 120:

U.S. APPLICATIONS		STATUS (Check One)		
U.S. Application Number	U.S. Filing Date	Patented	Pending	Abandoned
PCT APPLICATIONS DESIGNATING THE U.S.				
PCT Application No.	PCT Filing Date	U.S. Serial Numbers Assigned (if any)		
PCT/AU00/00181	March 10, 2000			

POWER OF ATTORNEY: As named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith: Stephen A. Becker, Reg. No. 26,527; John G. Bisbikis, Reg. No. 37,095; Daniel Bucca, Reg. No. 42,368; Kenneth L. Cage, Reg. No. 26,151; Jennifer Chen, Reg. No. 42,404; Bernard P. Codd, Reg. No. 46,429; Lawrence T. Cullen, Reg. No. 44,489; Paul Devinsky, Reg. No. 28,553; Margaret M. Duncan, Reg. No. 30,879; Shamita De. Etienne-Cummings, Reg. No. 46,072; Ramyar M. Farid, Reg. No. 46,692; Brian E. Ferguson, Reg. No. 36,801; Michael E. Fogarty, Reg. No. 36,139; John R. Fulsz, Reg. No. 37,327; Willem F. Gadiano, Reg. No. 37,136; Keith E. George, Reg. No. 34,111; John A. Hankins, Reg. No. 32,029; Eric J. Kraus, Reg. No. 36,190; Catherine Krupka, Reg. No. 46,227; Jack Q. Lever, Reg. No. 28,149; Raphael V. Lupo, Reg. No. 28,363; Burman Y. Mathis III, Reg. No. 44,907; Michael A. Messina, Reg. No. 33,424; Dawn L. Palmer, Reg. No. 41,236; Joseph H. Paquin, Jr., Reg. No. 31,647; Scott D. Paul, Reg. No. 42,984; William D. Pegg, Reg. No. 42,988; Robert L. Price, Reg. No. 22,685; Gene Z. Rubinson, Reg. No. 33,351; Joy Ann G. Serauskas, Reg. No. 27,952; David A. Spenard, Reg. No. 37,449; Arthur J. Steiner, Reg. No. 26,106; David L. Stewart, Reg. No. 37,578; Wesley Strickland, Reg. No. 44,363; Michael D. Switzer, Reg. No. 39,552; David M. Tennant, Reg. No. 48,362; Daniel S. Trainor, Reg. No. 43,959; Kelli N. Watson, Reg. No. 47,170; Cameron K. Weiffenbach, Reg. No. 44,488; Aaron Weisstuch, Reg. No. 41,567; Edward J. Wise, Reg. No. 34,523; Jeffrey A. Wolfer, Reg. No. 48,041; Alexander V. Yampolsky, Reg. No. 36,324; and Robert W. Zelnick, Reg. No. 38,976, all of McDermott, Will & Emery.

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I hereby declare that all statement made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signature of Inventor 201:	Signature of Inventor 202:	Signature of Inventor 203:
Date 16/01/02	Date	Date

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